Callus induction from leaves of different *paulownia* species and its plantlet regeneration

FAN Guo-qiang, ZHAI Xiao-qiao, ZHAI Cui-juan, BI Hui-tao
(Institute of Paulownia, Henan Agricultural University, Zhengzhou 450002, P R China)

Abstract: The experiment was carried out on five different species of *Paulownia* for callus induction from leaves. MS medium was adopted as basic medium, and from different combinations of NAA and BA the suitable media were determined for callus induction, bud differentiation, and root differentiation of five different species. MS+0.5NAA+4BA, MS+0.3NAA+2BA, MS+0.5NAA+4BA, MS+0.3NAA+6BA, and MS+0.3NAA+8BA were suitable media of callus inductions of leaves, respectively, for *Paulownia tomentosa, Paulownia australis, Paulownia fortunei, Paulownia elongata* and *P. tmentosa* x *P. fortunei*, and MS+0.3NAA+12BA, MS+0.3NAA+12BA, MS+0.5NAA+12BA, MS+0.5NAA+12BA, and MS+0.7NAA+12BA were suitable media for bud differentiation from leaf callus respectively for above five species. The rooting media was determined as 2MS+0.1NAA, 1/2MS+0.1NAA, 1/2MS, 1/2MS+0.3NAA, and 1/2MS+0.5NAA. These results provide reference data for breeding new fine varieties with different kinds of *Paulownia* protoplasts fusions.

Keywords: *Paulownia*; Callus induction; Plantlet regeneration; Medium; Hormone
CLC number: S792.43 **Document code:** A **Article ID:** 1007-662X(2001)04-0209-06

Introduction

With the rapid development of science and technology, scientists have gotten many species of regenerated plantlets from their calli or protoplasts of different tree species with biotechnology (Huang 1990; Jiang 1988; Tang *et al.* 1998; Wang 1988; Wang *et al.* 1991, 1993, 1995; Wei *et al.* 1991, 1992; Zhuge *et al.* 2000; Becwer *et al.* 1990; Gupta & Durzan 1987; Hakman & Frowke 1987; Laine & David 1990; Schenk & Hildebrant 1972; Von Arnold &Woodward 1988). Plantlet regenerations from calli were an indispensable step in the regenerations of forest tree protoplasts or transgenic protoplasts. *Paulownia* is an important timber tree in China, which is used widely as architeculture material and furniture because of their short rotation and rapid growth. Although regenerated plantlet from *P. tomentosa* protoplast was studied, only little plantlets from induced calli was obtained (Wei *et al.* 1991); besides, there existed some differences in callus inductions of different species of *Paulownia* plant protoplasts and their plantlet regenerations. Here the callus inductions of different species of *Paulownia* plant leaves and their regenerated plantlets were studied, which lays a foundation for protoplast fusions among different *Paulownia* plant leaves and their plantlet regeneration.

Foundation Item: This paper was supported by National Nature Science Foundation of China (No. 39870631) and Nature Science Foundation of Henan Province (No. 994011100).
Biography: FAN Guo-qiang (1964-), male, Ph. Doctor, Professor in Forestry Department of Henan Agriculture University, Zhengzhou 450002, P. R. China. E-mail: guoqiangf@263.net.
Received date: 2001-09-24
Responsible editor: Song Funan

Materials and methods

Materials

The materials are seeds of *Paulownia tomentosa* (Thunb.) Steud, *P. australis* Gong Tong, *P. fortunei* (Seem.) Hemsl, *P. elongata* S.Y. Hu, and *P. tomentosa x P. fortunei,* which were picked and collected in Zhengzhou City, Henan Province in September 1998.

Methods

Surface sterilization of those seeds and seedling incubation

Seeds were rinsed with 70% alcohol for 30 s and with 0.1% $HgCl_2$ for 5 min orderly, and then washed 5 times with enough sterile distilled water, finally put on the PC culture medium in incubator. Eighty days later, *Paulownia* seedlings with 6-8 leaves were obtained, and their leaves were used as the materials for inducing callus.

Medium for inducing callus from Paulownia leaves

1/2 MS, MS, B_5, N_6 and PC culture media modified adding some amount of NAA, 6-BA (briefly as BA), 25 $g \cdot L^{-1}$ sugar and 8 $g \cdot L^{-1}$ agar powder were used. First of all, the leaves of different species of *Paulownia* seedlings were cut into roughly pieces of 0.5cm x 1.0 cm (explants) and put in triangular bottles with media (10 bottles for each species, with 3 pieces of explants each bottle) for callus induction in darkness at temperature of 25±2 ℃. When the calli grew to a certain size, they were transferred into an incubator with light intensity of 1500 lx, light period of 16 h/d and temperature of 25±2 ℃. Twenty days later, we took a close observation upon the calli, calculated their induction rate, and determined the suitable culture media for leaves of different *Paulownia* species.

Medium for bud differentiation

Those induced calli were cut into about 1.0-cm^3 pieces and put on the MS media adding 0.1~1.1 $mg \cdot L^{-1}$ NAA (briefly as 0.1~1.1 NAA) and 2~12 $mg \cdot L^{-1}$ BA for bud differentiation in light condition. One piece was put in each bottle, and 20 bottles were taken for the leaves of each species of *Paulownia*. Twenty days later the induction rates were calculated.

Medium for rooting

The induced buds with certain height were cut at their stem bases and transferred on the rooting media, and then incubated in the light condition for root induction. Twenty days later we observed the root generation and chose the suitable rooting media of those induced buds.

Results and analysis

Callus induction of leaves for different species of *Paulownia*

The callus inductions of leaves of 5 different *Paulownia* species on different media were culture for 20 d (See Table 1). The induction rates of 5 species were highest on MS culture medium, and there were no calli induced on N_6 culture medium. That is to say, the callus induction from the leaves of different species of *Paulownia* had significant difference if the culture media were different, and MS culture medium could be taken as the basic one for inducing calli from leaves of *Paulownia*.

Table 1. Callus induction from the leaves of different *Paulownia* species on various media

Paulownia	Media	Number of mean explants	Mean Number of induced callus	Induction ratio
	1/2MS	28.2	5.0	17.7
	MS	26.4	12.8	46.8
P. tomentosa	B_5	27.6	7.0	25.1
	N_6	27.6	0	0
	PC	28.2	5.0	17.4
	1/2MS	26.4	5.4	20.5
	MS	26.4	10.6	40.2
P. australis	B_5	25.8	9.0	34.9
	N_6	28.2	0	0
	PC	25.8	2.4	9.3
	1/2MS	26.4	6.2	23.5
	MS	27.6	12.6	45.7
P. fortunei	B_5	26.4	11.0	41.7
	N_6	26.4	0	0
	PC	26.4	4.2	16.0
	1/2MS	26.4	4.6	17.4
	MS	27.6	9.8	35.5
P. elongata	B_5	25.2	6.0	23.8
	N_6	26.4	0	0
	PC	25.2	4.4	17.5
	1/2MS	25.8	7.0	27.1
	MS	26.4	12.8	48.5
P. tomentosa x *P. fortunei*	B_5	28.2	4.4	15.6
	N_6	26.4	0	0
	PC	26.4	9.6	36.4

Note: The values of the number of mean explants in above table were mean numbers of explants on the media with different hormone combinations (0.3NAA+1BA, 0.3NAA+3BA, 0.3NAA+5BA, 0.3NAA+7BA, 0.3NAA+9BA).

Callus induction of leaves on MS media

The callus induction of the leaves of different *Paulownia* species on MS media with different concentrations of hormones indicated that the induction rate declined with rising of BA concentrations when the NAA concentration was 0.1 $mg \cdot L^{-1}$ (Table 2). The change of induction rate rose first and then declined at NAA concentration of 0.3~0.7 $mg \cdot L^{-1}$. When NAA concentration was 0.9~1.1 $mg \cdot L^{-1}$ with combination of any BA concentrations, the induction rate became zero. These results clarified the callus induction of leaves of the 5 *Paulownia* species had similarity in heredity, but individual differences still existed in callus induction rates on the same culture medium. Moreover, callus of leaves of different *Paulownia* species could be divided into two catalogues: one was colorless transparent with brittle texture, the other was light-yellowish strumae with tight texture, and the later was used to proliferate buds. The callus inductions of leaves of different *Paulownia* species on MS culture media were shown in Fig.1 A~E.

Table 2. Calli induced from leaves of different *Paulownia* species on MS media

Concentrations of hormones /mg·L^{-1}	Induction rates				
	P. tomentosa	*P. australis*	*P. fortunei*	*P. elongata*	*P. tomentosa* x *P. fortunei*
0.1NAA+ 2BA	43.3	48.1	60.0	11.1	29.6
0.1NAA+ 4BA	18.5	26.7	37.5	0	20.0
0.1NAA+ 6BA	12.5	12.5	19.0	0	12.5
0.1NAA+ 8BA	0	0	0	0	0
0.1NAA+10BA	0	0	0	0	0
0.1NAA+12BA	0	0	0	0	0
0.3NAA+ 2BA	66.7	81.5	37.0	16.7	37.0
0.3NAA+ 4BA	83.3	66.7	88.3	47.6	50.8
0.3NAA+ 6BA	50.0	50.0	55.5	87.5	63.3
0.3NAA+ 8BA	14.2	23.8	23.3	33.3	86.6
0.3NAA+10BA	0	0	0	11.1	71.4
0.3NAA+12BA	0	0	0	0	48.1
0.5NAA+ 2BA	33.3	43.3	37.0	14.2	0
0.5NAA+ 4BA	92.5	74.0	85.7	83.3	41.7
0.5NAA+ 6BA	50.0	60.0	50.0	33.3	55.5
0.5NAA+ 8BA	12.5	55.6	12.0	11.1	66.7
0.5NAA+10BA	0	16.7	0	0	33.3
0.5NAA+12BA	0	0	0	0	0
0.7NAA+ 2BA	16.7	0	0	0	0
0.7NAA+ 4BA	55.6	47.6	23.8	20.0	12.5
0.7NAA+ 6BA	12.5	83.3	41.7	40.0	26.7
0.7NAA+ 8BA	0	29.6	50.0	18.5	28.6
0.7NAA+10BA	0	12.5	60.0	0	0
0.7NAA+12BA	0	0	16.6	0	0
0.9NAA+ 2BA	0	0	0	0	0
0.9NAA+ 4BA	18.5	0	11.1	0	0
0.9NAA+ 6BA	14.3	20.8	20.8	6.6	20.8
0.9NAA+ 8BA	0	14.3	33.3	12.5	0
0.9NAA+10BA	0	0	19.0	0	0
0.9NAA+12BA	0	0	0	0	0
1.1NAA+ 2BA	0	0	0	0	0
1.1NAA+ 4BA	0	0	0	0	0
1.1NAA+ 6BA	0	0	0	0	0
1.1NAA+ 8BA	0	0	0	0	0
1.1NAA+10BA	0	0	0	0	0
1.1NAA+12BA	0	0	0	0	0

Bud differentiation of leaves callus

From Table 3, it can be seen that the bud differentiation rates of leaves callus of *P. tomentosa* on MS medium changed with the hormone concentrations. The bud differentiation rate decrease with BA concentration rising at NAA concentration of 0.1 mg·L^{-1}, increased with BA concentration rising at NAA concentrations of 0.3~0.5 mg·L^{-1} and 0.7 mg·L^{-1}, but becomes zero at NAA concentrations of 0.9~1.1 mg·L^{-1}. When BA was 8 mg·L^{-1}, the bud differentiation rates declined gradually as NAA concentrations increasing; when BA was 10 mg·L^{-1} and 12 mg·L^{-1}, it tended to rise first and then decline with NAA concentration increasing. The bud differentiation rate was highest (94.7%) on MS+0.3 NAA+12 BA culture medium, so this medium was considered as the best culture medium for bud differentiation of *P. tomentosa*.

For *P. australis*, the bud differentiation rate of leaves callus decreased with BA concentrations rising (from 8 to 12 mg·L^{-1}) at NAA concentration of 0.1 mg·L^{-1}, but increased with BA concentration rising at NAA concentration of 0.3 mg·L^{-1}. When NAA concentrations was 0.5~0.7 mg·L^{-1} and BA was 8~12 mg·L^{-1}, the bud differentiation rate was lower. At NAA concentrations of 0.9~1.1 mg·L^{-1}, with three combinations of BA concentrations, the bud differentiation rate was always zero. By comparing these bud differentiation rates, it could be seen that MS +0.3 NAA+12 BA medium was suitable for leaves callus of *P. australis*.

Leaf callus of *P. fortunei* have higher bud differentiation rate on MS+0.3~0.5NAA+10~12BA medium. The bud dif-

ferentiation rates were always zero at NAA concentrations from 0.9 to 1.1 mg·L^{-1}, with three different BA concentrations. The MS+0.5 NAA+12 BA medium was the best culture medium for bud differentiation of leaf callus of *P. fortunei*, with a bud differentiation rate of 95%.

The bud differentiation rates of *P. elongata* were always zero at NAA concentrations of 0.1, 0.3, 1.1 mg·L^{-1}, respectively, with the combination of the three BA concentrations. The bud differentiation mainly presented on MS+0.5~0.9 NAA+12 BA media, with a differentiation rate of 90% on MS+0.5 NAA+12 BA medium, which could be chosen as the medium for bud differentiations from leaves callus of *P. elongata*.

Table 3. Buds induced from leaves callus of different *Paulownia* species on MS media with various hormone concentrations

Concentrations of hormones /m g·L^{-1}	*P. tomentosa*			*P. australis*			*P. fortunei*			*P. elongata*			*P. tomentosa* x *P. fortunei*		
	NC	B	IR	NC	B	IR	NC	Bds	IR	NC	B	IR	NC	B	IR
0.1NAA+ 8BA	20	5	25.0	19	3	15.6	20	5	25.0	20	0	0	20	0	0
0.1NAA+10BA	20	3	15.0	20	2	10.0	20	2	10.0	19	0	0	20	0	0
0.INAA+12BA	19	2	10.5	18	0	0	19	0	0	20	0	0	19	0	0
0.3NAA+ 8BA	20	3	15.0	20	10	50.0	20	0	0	20	0	0	18	0	0
0.3NAA+10BA	18	15	83.3	20	15	75.0	18	10	55.6	18	0	0	20	5	25.0
0.3NAA+12BA	19	18	94.7	19	19	100.0	20	19	95.0	19	0	0	20	8	40.0
0.5NAA+ 8BA	20	0	0	18	0	0	19	0	0	20	0	0	20	0	0
0.5NAA+10BA	19	8	42.1	20	2	10.0	20	12	60.0	17	10	58.8	19	6	31.6
0.5NAA+12BA	18	14	77.8	20	5	25.0	18	18	100.0	20	18	90.0	17	10	58.8
0.7NAA+ 8BA	20	0	0	20	0	0	20	0	0	19	0	0	20	0	0
0.7NAA+10BA	19	2	10.5	19	0	0	20	5	25.0	20	13	65.0	18	10	55.5
0.7NAA+12BA	17	5	29.4	20	2	10.0	19	10	52.6	20	15	75.0	20	18	90.0
0.9NAA+ 8BA	19	0	0	20	0	0	20	0	0	18	0	0	20	0	0
0.9NAA+10BA	20	0	0	20	0	0	18	0	0	20	8	40.0	19	4	21.1
0.9NAA+12BA	19	0	0	20	0	0	20	0	0	19	3	15.6	17	6	35.3
1.1NAA+ 8BA	20	0	0	20	0	0	17	0	0	17	0	0	18	0	0
1.1NAA+10BA	20	0	0	19	0	0	20	0	0	20	0	0	20	0	0
1.INAA+12BA	18	0	0	18	0	0	20	0	0	19	0	0	20	0	0

Note: NC: Number of callus; B: buds; IC: induction ratio.

The leaves callus of *P. tomentosa* x *P. fortunei* produced bud differentiation on MS+0.3~0.9 NAA+10~12 BA media, with a differentiation rate of 90% on MS+0.7 NAA+12 BA medium. But the differentiation rate was zero when NAA concentrations were 0.1 mg·L^{-1} and 1.1 mg·L^{-1}, combining with any BA concentration respectively. For this reason, MS+0.7 NAA+12 BA medium was chosen as the suitable medium for the bud differentiation from leaves callus of *P. tomentosa* x *P. fortunei*.

Differentiated buds from leaves callus of 5 *Paulownia* species were shown in Fig.1 (F~J).

Root differentiation of differentiated buds

Rooting of differentiated buds on 1/2MS (modified) media with various concentrations of NAA was shown in Table 4 and Fig.1 (K~P). The rooting rates of the differentiated buds for *P. australis, P. fortunei, P. elongata* and *P. tomentosa x P. fortunei* were all up to 100% on the media with 0, 0.1, 0.3, and 0.5 mg·L^{-1} NAA, but there existed difference in number and length of root for different species on different concentrations of NAA. Based on this, the 1/2 MS+0.1 NAA, 1/2 MS+0.1 NAA, 1/2MS, 1/2MS+0.5 NAA, and 1/2MS+0.3 NAA media were chosen as the rooting media respectively for *P. tomentosa, P. australis. P. fortunei. P. elongata and P. tomentosa x P. fortunei*, respectively.

Table 4. Rooting of different *Paulownia* plantlet on 1/2MS media with various NAA concentrations

Paulownia plants	NAA concentrations	Rooting percentage	Number of mean roots	Mean length of roots
P. tomentosa	0.0	100	3	2.5
	0.1	100	5	3.0
	0.3	100	4	2.1
	0.5	100	2	1.8
P. australis	0.0	100	4	2.5
	0.1	100	8	3.0
	0.3	100	5	2.0
	0.5	100	3	1.5
P. fortunei	0.0	100	8	3.5
	0.1	100	6	2.4
	0.3	100	4	2.0
	0.5	100	2	1.8
P. elongata	0.0	100	5	2.5
	0.1	100	3	2.4
	0.3	100	4	1.7
	0.5	100	8	2.7
P. tomentosa x *P. fortunei*	0.0	100	3	1.8
	0.1	100	5	2.6
	0.3	100	8	3.1
	0.5	100	4	2.5

Discussions

The suitable culture media of different tissues or organs of plant to induce callus differed with the plants of different genus, family and species. Calli from tissues or organs of rice and wheat were induced on N_6 culture medium with proper concentration of hormones (Chen 1980). Calli from leaves of *Platanus occidenalis* were induced on MS culture media (Sun 1982, 1991). In this study, we found that the callus induction rates of the leaves of *P. tomentosa, P. australis, P. fortunei, P. elongata*, and *P. tomentosa x P. fortunei* were the highest at MS culture medium; lower at 1/2MS, B_5 and PC; and zero at N_6. Therefore, MS could be chosen as the basic culture medium to induce calli from the leaves of the five species of *Paulownia* plants, which was consistent with results reported before (He & Dai 1999; Jiang 1988; Shi 1995). The differences of plant species and their metabolic activity, especially hormone contents, resulted in requiring different hormone and concentration on culture media for callus induction from different species of plant tissues or organs (Huang 1990; Wei *et al.* 1991, 1992; Emily 1991; Julie 1986; Sticklen 1986; Von Arnold & Woodward 1988). Although the five species in this study were the plants in same genus, and especially *P. australis, P. elongata and P. tomentosa x P. fortunei* were all the hybrid varieties of *P. tomentosa* and *P. fortunei* (Fan *et al.* 2001), due to a long-term natural evolution, there exists differences in physical properties, biochemical metabolic level as well as hormone contents among them, and maybe this is main reason for that the most suitable media are different for callus inductions of the five species.

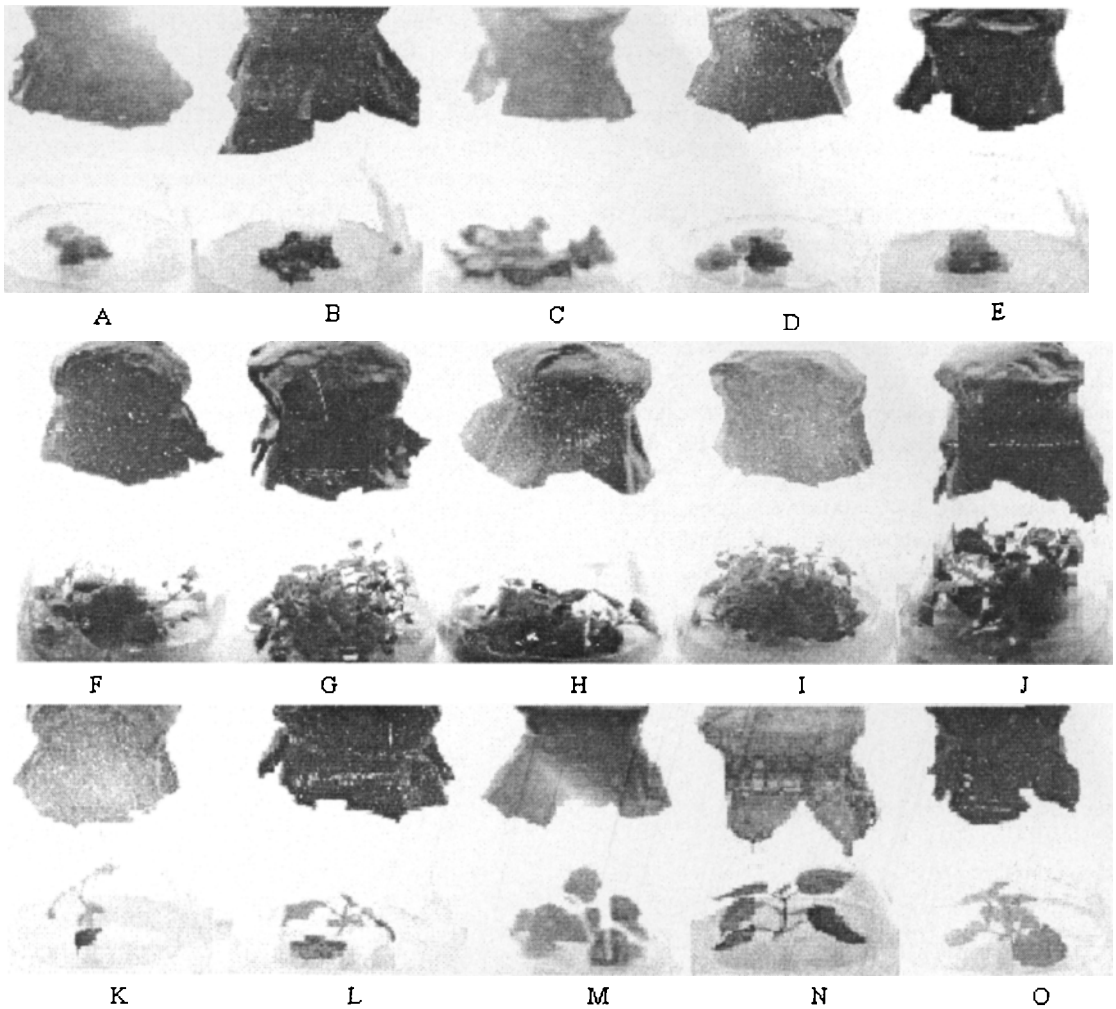

Fig. 1 Callus inductions of leaves of different species of *Paulownia* and their plant regenerations

A~E Callus of *P. tomentosa, P. australis, P. fortunei, P. elongata, P. tomentosa* × *P. fortunei*

F~J Proliferated buds from callus of *P. tomentosa, P. australis, P. fortunei, P. elongata, P. tomentosa* × *P. fortunei*

K~O Regenerated plants of *P. tomentosa, P. australis, P. fortunei, P. elongata, P. tomentosa* × *P. fortunei*

References

Becwar, M.R., Nagmani, R. *et al.* 1990. Initiation of embryogeni cultures and somatic embryos development in loblolly pine [J]. Can. J. For. Res., **20**: 810-81.

Chen Ying. 1980. Rice pollen culture and plant regeneration [J]. Scientia Snicae, **7**(1): 46-54.

Emily, E.C. 1991. Plantlet multiplication from white pine (*Pinus strobus* L) embryos in vitro: bud induction and rooting [J]. Plant Cell, Tissue and Organ Culture, **26**:107-114.

Fan Guoqiang, Peng Haifeng. 2001. Protein diversity of Paulownia plant leaves and clusters [J]. Journal of Forestry Research, **12**(1): 21-24.

Gupta, P.K., Durzan, D.J. 1987. Biotechnology of somatic polyembryogenesis and plantlet regeneration in loblolly pine [J]. Biotechnology, **5**: 147-151.

Hakman, I., Fowke, L.C. 1987. Somatic embryogenesis in *Picea glaua* and *Picea mariana* [J]. An. J. Bot., **65**: 656-659.

Hakman, I., Fowke, L. C. *et al.* 1985. The development of somatic embryos on tissue cultures initiated from immature embryos of *Picea abies* [J]. Plant Sci., **38**: 53-59.

He Po, Dai Qun, He Fangtiny. 1999. Study on genetic transformation of *Paulownia* [J]. Journal of Shandong University, **34**(3): 332-338.

Huang Minren. 1990. Present research of forestry protoplast culture [J]. Plant Physiology communication, (2): 75-78.

Jiang Guowu. 1988. Economic plant tissue culture [M]. Peking: Science Press, p216-220.

Julie, A. 1986. Techniques for enhanced release of leaf protoplast in *Populus* [J]. Plant Cell Reports, **5**: 284-287.

Laine, E. David, A. 1990. Somatic embryogenesis in immature embryos and protoplast of *Pinus caribaea* [J]. Plant Sci., **69**: 215-224.

Schenk, R.U., Hildebrant, A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures [J]. Can. J. Bot., **50**: 199-204.

Shi Shizheng. 1995. Primary study on *Paulownia* tissue culture [J]. Jiangsu Forestry Science and Technology, **22**(3): 20-22.

Sticklen, M.B. 1986. Shoot regeneration from protoplasts of Ulmus × Pioneer [J]. Plant Science, **47**: 29-34.

Sun Yongru. 1982. Somatic hybridization between *Nicotiana lauca* and *Pharbitis nil* and its plant regeneration. Genetica Snicae, **9**(4): 284-288.

Sun Yongru. 1991. Plant protoplast culture [M]. Beijing: Science Press, p116-122.

Tang Wei. 1998. Study on embryogenic callus induction and plant regeneration in loblolly pine [J]. Scientia Silvae Snicae, **34**(3): 115-119.

Von Arnold, S. Woodward, S. 1988. Organpgenesis and embryogenesis in mature embryos of *Picea sitchensis* [J]. Tree Physiol., **4**: 291-300.

Wang Shanping. 1988. Woody plant protoplast culture [J]. Journal of Cytobiology, **11**: 14-18.

Wang Ying, Huang Minren, Chen Daoming, *et al.*. 1991. A study on cell suspension culture and somatic embryogenesis of *Populus simonii* [J]. Journal of Nanjing Forestry University, **15**(3): 31-36.

Wang Ying, Huang Minren. 1993. Progress in research of forest protoplast culture [J]. Journal of Nanjing Forestry University, **17**(7): 91-95.

Wang Ying, Huang Minren. 1995. Plants regenerated from protoplasts of *Populus simonii* and variations in its isoenzyme [J]. Scientia Silvae Snicae, **31**(4).

Wei Zhiming, Xu Nong. 1991. Isolation of platanus leaf protoplast and its plant regeneration [J]. Acta Botanica Sinica, **33**(11): 813-818.

Wei Zhiming, *et al.* 1991. Plant regeneration from *Paulownia tomentosa* protoplast [J]. Plant Tissue Culture, **8**(2): 111-113.

Wei Zhiming. 1992. Plantlet regeneration from protoplasts of Mulberry leaves [J]. Plant Physiology Communication, **28**(4): 248-249.

Zhuge Qiang, Huang Minren. 2000. A study on poplar spmatic hybridization [J]. Journal of Nanjing Forestry University, **23**(2): 6-10.